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TRANSACTIONS

Über die Blutamylase und Körperkraft bei einiger Rassen der Seidenraupen.

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Zur Verbesserung des Seidenbaues hat die japanische Regierung im vorigen Jahre einige neue Rassen ausbilden und unter die Seidenraupenzüchter verteilen lassen. Es ist daher für uns dringend notwendig, die Eigentümlichkeiten dieser Rassen näher zu erkennen. Zunächst haben wir die amylolytische Tätigkeit des Blutes und die Widerstandsfähigkeit der Raupen gegen ungünstige Ernährungsbedingungen untersucht; denn die erstere ist, nach unseren Forschungen[1][2], eine besonders bedeutende Funktion bei den Stoffwechselvorgängen im Körper und die letztere ohne Zweifel eine sehr wichtige Eigenschaft bei der praktischen Seidenzucht.

1. BLUTAMYLASE.

Um die Amylasewirksamkeit des Larvenblutes zu bestimmen, unternahmen wir im Herbst 1935 die Auffütterung von Seidenraupen dreier verschiedener Rassen: Japan-8, China-17, und Europa-19. Zur Ermittlung der Enzymwirkung wurden nur die gesunden Larven dieser Rassen benutzt. Die Bestimmungsmethode war folgende: Es wurden 20 ccm 3%ige stärkelösung mit 3 ccm 0,2 m-Phosphatpuffer nach Sörensen (pH 6,8), 1,6 ccm 0,85%iger NaCl-Lösung und 0,4 ccm Blute versetzt. Dieser Ansatz, mit Toluol überschichtet, kam bei 30° in den Brutofen. Nach 24 Stunden wurden die reduzierenden Zucker mittels der Verfahrens von Bertrand bestimmt. Die Zahlen in der Tabelle 1 bedeuten ccm 0,5 %ige $KMnO_4$-Lösung.

Tabelle 1.

Tage im V. Lebensalter	Japan-8		China-17		Europa-19	
	♀	♂	♀	♂	♀	♂
1	4,3	5,2	——	——	9,2	8,6
2	4,7	5,6	——	——	11,9	11,8
3	5,7	6,4	8,0	8,4	12,0	11,3
4	7,1	8,1	8,5	8,6	12,5	11,3
5	8,5	7,0	9,2	8,7	13,5	11,5
6	7,7	7,6	9,4	7,8	12,3	10,6
7	9,5	7,7	9,8	7,0	11,6	11,1
8	9,5	7,9	8,3	8,0	11,9	10,7
9	9,3	7,9	10,1	9,4	12,4	12,4

2. Körperkraft.

In der Absicht, die Körperstärke der drei oben erwähnten Rassen genau zu vergleichen, wurden die Raupen während des fünften Lebensalters in ungünstiger Umgehung, d. h. unter ungenügender Ventilation und etwas höherer Temperatur und Feuchtigkeit als normale aufgezogen. Durch Zählen der toten Würmer konnten wir die Widerstandskraft des Larvenkörpers dieser erkennen. In Tabelle 2 sind die gewonnenen Ergebnisse zusammengestellt.

Tabelle 2.

Tage im V. Lebensalter	Japan-8		China-17		Europa-19	
	♀	♂	♀	♂	♀	♂
1	——	——	——	——	——	——
2	——	1	——	——	2	2
3	5	9	——	——	2	4
4	5	4	——	——	1	1
5	2	8	——	——	4	2
6	7	3	4	——	1	2
7	6	8	7	1	2	3
8	10	5	——	4	7	1
9	7	3	2	3	——	——
Summe	42	41	13	8	19	15

Zusammenfassung.

(1) Es wurden die Amylasetätigkeit des Blutes und die Körperstärke der drei neuen Rassen untersucht, welche im vorigen Jahre durch die japanische Regierung ausgewählt wurden.

(2) Während des fünften Lebensalters der Larve nimmt der Amylasegehalt des Blutes täglich zu, und zwar bei der Rasse Japan-8 etwas plötzlich, aber bei China-17 und Europa-19 sehr langsam.

(3) Bei Europa-19 ist die Aktivität der Blutamylase bei den Weibchen stets starker als bei den Männchen. Bei Japan-8 nnd China-17 ist sie aber im ersten Halbstadium des fünften Alters bei den Weibchen schwächer als bei den Männchen, im letzten Halbstadium dagegen bei den Männchen schwächer.

(4) Unter den drei Rassen ist die Amylase von Europa-19 am stärksten; Japan-8 und China-17 besitzen ungefähr gleichen Gehalt des Blutes an Amylase.

(5) Die Widerstandsfahigkeit der Raupen gegen ungünstige Züchtung ist bei China-17 am grössten und bei Japan-8 am kleinsten.

(6) Bei den von uns gewählten Züchtungsbedingungen starben etwa 80% der Raupen der japanischen Rasse, 40% der europäischen, aber nur 20 % der chinesischen.

(7) Die Körperkraft des Weibchens ist bei diesen drei Rassen immer etwas schwächer als die des Männchens.

Herrn Prof. Okuda möchten wir für die lebhafte Förderung unserer Arbeit herzlich danken.

Literatur.

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(2) K. Yamafuji: Z. physiol. Chem. im Druck.

Biochemical Studies on "Miso", Fermented Soy-been Paste. Part IV.

On the Effect of Cystine upon the Nutritive Value of "Miso-protein" when fed to Albino Rats as a Supplement to Rice.

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Introduction

The author[1] has recently found that an adequate amount of cystine increases the supplementary nutritive value of "Miso-protein", but the amount to be given largely depends upon the age of the animal, namely, for young rats, until sexual maturity, the most satisfactory amount was found to about 0.1% of the diet.

According to A. C. Curtis, L. H. Newbourgh and F. H. Thomas[2], rats fed on 18% casein diet which supplied 0.047% free cystine grew well. As the cystine content of casein was found to be 0.17%[3], the diet contained 0.078% cystine and this diet was favorable to healthy growth. Similarly, cystine accounting to 0.43% of food protein may favorably influence the growth and nitrogen retention of the rat. According to the experiment of U. Suzuki, Y. Okuda and Y. Matsuyama[4] the rats receiving a 10～18% cod-fish protein diet, exhibited very good growth. As the cystine content of the protein was reported to be 0.67%[5], an adequate amount of cystine was calculated to be 0.06～0.121% of the diet, or 0.61～0.67% of the protein.

In the preceeding experiment, however, young rats fed on the "Miso" diet have improved in rate of growth, so it may be held that "Miso" contains some cystine. Since the animals were restricted to the diets containing about 10% protein, in order to reduce the amount of NaCl, it is probable that the diets were deficient in cystine, but it is uncertain whether or not the "Miso-protein" is qualitatively poor in cystine.

From this point of view, it is very important to estimate the cystine in "Miso", owing to the caramelized brown colour, which developed during the fermentation of "Miso", its presence remains to be proved. Fortunately, the present author observed that the characteristic brown color is hydrolysed with concentrated hydrochloric acid and then can be easily decolorized with a small amount of charcoal. Hereupon, Y. Okuda's method[6] for the estimation of free cystine was applied to this case and the result was compared with Folin and Looney's method[7][8].

During the feeding experiment it was found that the urine of the rats receiving cystine diet gave a positive Folin and looney's reaction, which seemed to indicate the presence of a large amount of cystine.

The purpose of the present investigation was to ascertain the absolute amount of cystine in the "Miso" diets as well as the adequacy of cystin in the "Miso-protein". Moreover, in order to determine whether any of the primary constituents were responsible for the amount of cystine administration, cystine in urine, kidneys and liver has been colorimetrically estimated.

Experimental.

I. Determination of Cystine in "Miso".

(A) Y. Okuda's method : —

For acid hydrolysis, 25 g of "Miso" freshly drawn were boiled for 12 ~ 20 hours with 125 c.c. of concentrated hydrochloric acid. This was then filtered by a nutsche, washed with hot water, made up to 250 c.c. and used for determination. The following reagents were used in the experiment.

(1) 5% of KI.

(2) exactly 4% HCl.

(3) 20% NaOH.

(4) M/300 KIO_3; 2.14 g of potassium iodate were dissolved in 3 l. of exactly 2% HCl.

(5) Cystine solution in 2% HCl ; 1 c.c. of the solution should be contain 1 mg. of l-cystine.

To 25 c.c. (or 35 ~ 40 c.c.) of the sample, were added 10 c.c. of a standard cystine solution and 20% NaOH, and brought up to 50 c.c. with water. After ascertaining the solution to be 4.0% HCl, 0.5 g of Merck's pure animal charcoal were added. The solution was then boiled for exactly one minute and allowed to stand for 10 minutes. Then it was filtered by suction, washed with about 5 c.c. of hot water, and after cooling to 20, the solution was reduced by 0.5 g of zine powder, and shaken several times during a period of 30 minutes. The filtrate was filled up to 100 c.c., well mixed and after confirming the fact that the solution was exactly 2% HCl, 20 c.c. of the hydrolysate was poured into a dry Erlenmeyer flask and to the mixture was added 5 c.c. of both (1) and (2). Immediately, it was titrated by M/300 of potassium iodate until the yellow color fades in exactly one minute. The filtration should place in exactly two minutes, as the color starts to increase with the lapse of time.

It is important to note that cystine is more or less adsorbed by charcoal. Accordingly, the reading was revised by substracting the standard from unknown plus standard. All steps in the determination should be carried out in the same manner both standard and unknown plus standard. As a differ-

ence of the temperature of the solution on titration considerablly influences the amount of potassium iodate which acts on cystine, c.c. of M/300 of potassium iodate was obtained as follows:

0.0098 g of cystine is equivalent to 4.55 c.c. of M/300 of KIO_3 at 23°, then

cystine (g) $= 0.0098/4.55 \times Q$

$= 0.00216 \times Q$

Similary, 0.0098 g of cystine is equivalent to 5.10 c.c. of M/300 of KIO_3 at 25°, and then cystine (g) $= 0.0098/5.1 \times Q$

$= 0.0019 \times Q$ Q……Reading

(B) Folin and Looney's Method: —

In the presence of sodium sulfite, cystine produces a deep blue color with phospho-tungstic acid. Though other reducing substances give the same color with tungstic acid, with sodium sulfite the color is not changed. Therefore, the amount of cystine was colorimetrically estimated by the increase of the color intensity. For estimation the following reagents were used.: —

(1) Standard cystine solution; in 1 c.c. of 5% sulphuric acid contains 0.5 mg cystine.

(2) Standard sodium carbonate solution.

(3) 20% of sodium sulfite.

(4) 20% of lithium sulfate.

(5) Folin and Deni's uric acid reagent; to 80 c.c. of 85% phosphoric acid (S.G.=1.17) were added 100 g of sodium phospho-tungstate and 700 c.c. of water. This was boiled in a water-bath for 20 hours with a reversible coolor, and after cooling, filled up to 1 L.

For the determination, three 100 c.c. volumetric flask were required. In the first volumetric flask 1 c.c. of standard solution containing 0.5 mg of cystine, 20 c.c. of standard sodium carbonate, 10 c.c. of 20% sodium sulfite, and 1 c.c. of 20% lithium sulfate were added and the solution was well mixed. In the second and in the third flask 1~10 c.c. of the sample were placed and then treated in the same manner as above, but in the third flask the sodium sulfite solution was not added. Now to each flask was added 3 c.c. of uric reagent, mixed by shaking vigorously, and let to stand for exactly five minutes. Subsequently, these solutions were filled up to the mark with water, and the readings were taken, within 8 minutes (room temperature at 10~16) after adding uric reagent and keeping the standard at 20 mm of Duboscq colorimeter. It was clear that the reading of the unknown should be taken within the range of from 13~30 mm, Since the greater difference of reading between the standard and the unknown the greater the discrepancy. The determination adopted for 25 c.c. of "Shiromiso" hydrolysate was as follows:

(1) 0.25 mg of standard cystine set at 20 mm.

(2) Reading of 2.5 c.c. of the sample with sodium sulfite was 13.9 mm,

then cystine (mg)$=20\times0.25/13.9=0.36$.

(3) Reading of 2.5 c.c. of the sample was 26.9 mm. then cystine (mg) $=20\times0.25/26.0=0.192$, therefore, the cystine in 2.5 c.c. of the sample$=0.36-0.192=0.168$ mg. The cystine content of a different kind of "Miso" is as follows :

TABLE I. Cystine Content in "Miso"

"Miso"	Time required for hydrolysis	Water	Cystine by Okuda's method	Cystine by Folin-Looney's method
"Shiromiso"*	12	50.26	67.4	71.5
	20	50.26	61.0	68.7
	Average	50.26	64.2	70.1
"Yedomiso"*	12	45.20	42.7	85.6
	20	45.20	48.0	84.8
	Average	45.20	45.4	85.2
"Sendaimiso"*	12	50.13	49.4	86.6
	20	50.13	52.3	87.0
	Average	50.13	50,9	86.8
"Hatchomiso"**	12	41.85	98.3	103.2
	20	41.85	88.5	115.5
	Average	41.85	93.4	109.9

(Cystine mg in 100 g of fresh substance.)
*.....Preparation of the Suehiro-miso Manufacturing Co.
**.....Preparation of the Iseya-miso Manufacturing Co.

II. Determination of Cystine in Polished Rice and Soy-beans.

Both to 25 g of polished rice and to 10 g of soy-beans were added six times the amount of concentrated hydrochloric acid. This was boiled for 20 hours on a sand-bath, filtered by suction, washed with hot water, and the former filled up to 500 c.c. the latter to 250 c.c.

The amount of cystine determined by Okuda's and by Folin and Looney's methods is then shown in the following table :

TABLE II. Cystine Content of Polyshed Rice and Soy-beans.
(Cystine mg in 100 g of substance)

	Water	Cystine by Okuda's method	Cystine by Folin-Looney's method
Polished rice used for Preparation of "Miso"	9.84	50.4	65.0
Polished rice used for the experiment of Vitamin B	10.64	37.8	59.3

Polished rice used for the feeding exp. of "Miso"	8.25	55.2	83.6
Soy-beans used for preparation of "Miso"	10.5	271.5	277.8
25 g Polished rice + 25 g Soy-beans	——	80.7	100.4

From cystine contents in "Miso" and polished rice determined by Okuda's method, the amount of cystine to be contained in the "Miso" diets, which were used in the preceeding experiment, was calculated :

TABLE III. Amount of Cystine in Diets.

	"Yedomiso" diet	"Sendaimiso" diet	"Hatchomiso" diet
"Miso" in 100 g of the diet.	40.2	39.3	22.4
Cystine (mg) in the above "Miso".	18.3	20.0	20.9
Polished rice in 100 g of the diet.	73.2	73.2	73.2
Cystine (mg) in the above Polished rice.	43.7	43.7	43.7
Total cystine (mg)	62.0	63.7	64.6

As indicated in the above table, it was found that "yedomiso", "Sendaimiso" and "hatchomiso" diets contained cystine in the proportion of 62.0, 63.7 and 64,6 mg respectively, amounting to 0.062～0.065% of the diet, or to 0.57 % of the protein. Based on th fact that the cystine content of the diet should be sufficient for the normal growth of white rats at 0.43～0.67% of the diet-protein, or that 0.037—0.121 g of cystine in 100 g of diet shows good growth, it may be justifiable conclusion that polished rice with "miso" alone gave a relatively poor growth, which may have been largely due to the imposibility of eating a sufficient amount of cystine rather than to the defectiveness of the protein constituents, since each diet consisted of 10.76% protein.

III. Excretion and Deposition of Cystine in Animals on a Cystine Diet.

Considering the fact that the urine of rats fed on the "Miso" diet supplemented by free cystine were strongly positive on Folin-Looney's reaction, the urine was collected by putting an enamelled plate covering with copper net, in the cage of the young rats. After 24 hours the urine was weighed, filtered, made up to 100 c.c. with water, after which 10 c.c. were used for Folin-Looney's colorimetric determination. Although this reaction is not specific for free cystine it is probable that dialanyl-cystine, glutathion[10], insulin[11], ergothioneine[12], and a solution of wool in HCl[13], the presence of an appreciable amount of cystine or allied substances. Hence, the results of the determi-

nation were indicated as Folin–Looney's value being expressed by crystine mg. Concerning the presence of thiocyanic acid[14], a number of specimens of the urine gave a negative reaction.

TABLE IV. Folin-Looney's Value of the Urine of Rats fed on the "Miso" Diets.

Experimental diet	Number of rat	Total body wts. (g)	Urine exc. per day (g)	Cystine exc. per day (mg)	Cystine exc. per 100 g of body wts. (mg)	NaCl exc. per day (g)	NaCl exc. per day per 100 g body wts (g)
"Yedomiso"	3	304	30.1	1.1	0.36	1.05	0.34
"Yedomiso"+ 0.5% Cystine	3	393	35.8	1.8	0.50	1.29	0.33
"Sendaimiso"	3	286	28.6	0.9	0.31	0.91	0.32
"Sendaimiso"+ 0.1% Cystine	3	401	30.8	1.4	0.35	1.38	0.34
"Sendaimiso"+ 0.5% Cystine	3	445	41.6	1.5	0.34	1.44	0.32
"Sendaimiso"+ 1.0% Cystine	3	333	40.9	4.5	1.35	1.19	0.36
"Hatchomiso"	3	302	35.0	1.5	0.50	1.05	0.35
"Hatchomiso"+ 0.5% Cystine	3	418	45.7	2.6	0.63	1.39	0.33
A. Patrogen	2	400	25.0	0.4	0.10	0.09	0.02
B. Patrogen	2	438	28.0	0.5	0.11	0.12	0.03

As indicated in Table IV it was shown that Folin–Looney's value of the urine was much influenced by the amount of cystine administrated to the rats, their value being estimated as follows:

"Miso" diets were 0.31～0.50 (cystine mg excreted per day per 100 g of body weight), "Miso" plus 0.5% cystine 0.34～0.62, and "Miso" plus 1.0% cystine 1.35 mg while Patrogen, 8.5 g of which was added per 100 g of body weight, was 0.10=0.11 mg.

The amount of NaCl excreted per 100 g of body weight was also determined, rats placed on a "Miso" diet being found to have excreted 0.32～0.36 g per day while those rats fed on Patrogen 0.02～0.03 g.

Further it was found that in cases of cystinuria the amount of cystine in kidneys and liver was abnormally high and was proportional to the hypertrophy of the kidneys, probably due either to the increased administration of cystine or to the excess of NaCl. The result of the determination of cystine

was indicated in the following table.

TABLE V. Folin-Looney's Value of Kidneys and Livers.

Experimental diet	No. of rat	Final body wts (g)	Kidneys (g)	Cystine in Kidneys (mg)	Liver (g)	Cystine in liver (mg)
Polished rice +"Yedomiso"	53	99	1.21	——	5.25	——
	54	108	0.75	0.58	3.87	1.05
	55	122	0.80	0.66	4.16	1.38
	Average	109.7	0.78	0.62	4.42	1.22
Polished rice +"Yedomiso" +0.5% cystine	56	127	1.15	0.70	4.92	——
	57	133	1.17	0.67	5.05	1.48
	58	142	1.63	0.81	5.31	1.50
	Average	134	1.32	0.73	5.06	1.49
Polished rice +"Sendaimiso"	59	111	0.90	——	3.98	——
	60	115	0.70	0.65	3.64	1.45
	61	131	0.91	0·72	4.12	1.60
	Average	119.0	0.85	0.69	3.91	1.53
Polished rice +"Sendaimiso" 0.1% cystine	62	124	1.25	0.70	4.80	1.39
	63	152	1.43	0.79	5.38	1.95
	64	129	1.20	0.58	5.23	1.62
	Average	135.0	1.29	0.69	5.31	1.65
Polished rice +"Sendaimiso" +0.5% Cystine	65	134	1.38	0.75	4.99	1.69
	66	142	1.51	0.80	5.21	1.87
	67	146	1.75	0.96	5.29	2.05
	Average	140.7	1.55	0.83	5.16	1.87
Polished rice +"Sendaimiso" +1.0% cystine	68	103	——	——	——	——
	69	107	1.39	0.97	4.86	1.78
	70	134	1.64	0.98	4.97	2.35
	Average	114.7	1.52	0.98	4.92	2.07
Polished rice +"Hatchomiso"	71	122	1.05	——	5.12	——
	72	112	0.82	0.81	4.05	1.40
	73	129	1.01	0.85	4.92	1.56
	Average	121.0	0.92	0.83	4.70	1.48
Polished rice +"Hatchomiso" +0.5% cystine	74	137	1.43	0.86	5.10	1.97
	75	134	——	——	——	——
	76	152	1.65	1.20	5.39	2.60
	Average	141.1	1.54	1.03	5.24	2.15

The weight of the kidneys differs according to the cystine administered, varying from 0.78 to 1.55 g with a corresponding rise in Folin-Looney's value which made obvious variation from 0.62 to 1.03. On the contrary the weight

of the liver was slightly less than normal. The deposition of cystine in the liver differed according to the amount of cystine administered, ranging from 1.22 to 2.15 mg.

In connection with the cystinuric investigation several specimens of human urine were examined but no suggestive evidence in cystine metabolism was obtained, but it was found that Folin-Looney's value in the urine is closely related to the sex, age, diet and physiological condition of the subject.

TABLE VI. Folin-Looney's Value of Human Urine.
(Cystine mg in 100 c.c. of Urine)

Kind of urine	Time of excretion	Color tone	Folin-Looney's value (mg)	Okuda's method cystine (mg)	c.c. of $AgNO_3$ titrating 5 c.c. of Urine
A	10 a.m.	++	18.4	—	12.1
B	10 a.m.	++	15.6	—	18.8
C	3 p.m.	+	4.8	—	12.0
D	4 p.m.	++	5.6	—	13.3
E	2 p.m.	+	7.1	3.86	10.6
F	10 a.m.	+	11.8	4.54	12.4
G	3 p.m.	+	12.0	3.56	9.5
H	5 month old child, 8 a.m colorless		0	—	0.8
I	Maternity case, 8 a.m. almost colorless		0	—	7.9

The administration of "miso", consisting of polished rice, soy beans and salt to an animal, produces an accumulation of a large amount of potassium salts in the body, as may be reduced by a corresponding amount of sodium salt.

TABLE VII. Potassium, Sodium and Calcium salts in Rice[15] and in Soy-beans.
(g in 1000 g of dry matter)

	K_2O	Na_2O	CaO
Rice	2.0	0.4	0.3
Polished rice	1.4	0.1	0.2
Soy-beans	12.6	0.3	1.7

In order to determine whether these salts in a large amount are responsible for hypertrophy of the kindneys, or whether the animal is not affected by an excess of NaCl, the next experiment was carried out.

Now two groups of white rats, each consisting of two rats, were used. For 35 days the first group was placed on the "Sendaimiso" diet supplemented with 1.0% cystine, and the second was placed on the same diet supplemented with *d*-glutamic acid (m p 196～198 uncor.) and containing the same amount of nitrogen. The latter group did not differ much from the first to indicate the appreciable effect of glutamic acid, although of a short duration

of the feeding. After 35 days feeding the urine of both groups was determined by Folin-Looney's test and then on the 39 th day the diets were exchanged (Chart IV), the first group receiving the diet supplemented with glutamic acid, while the second received the cystine diet. On the 46 th day to each

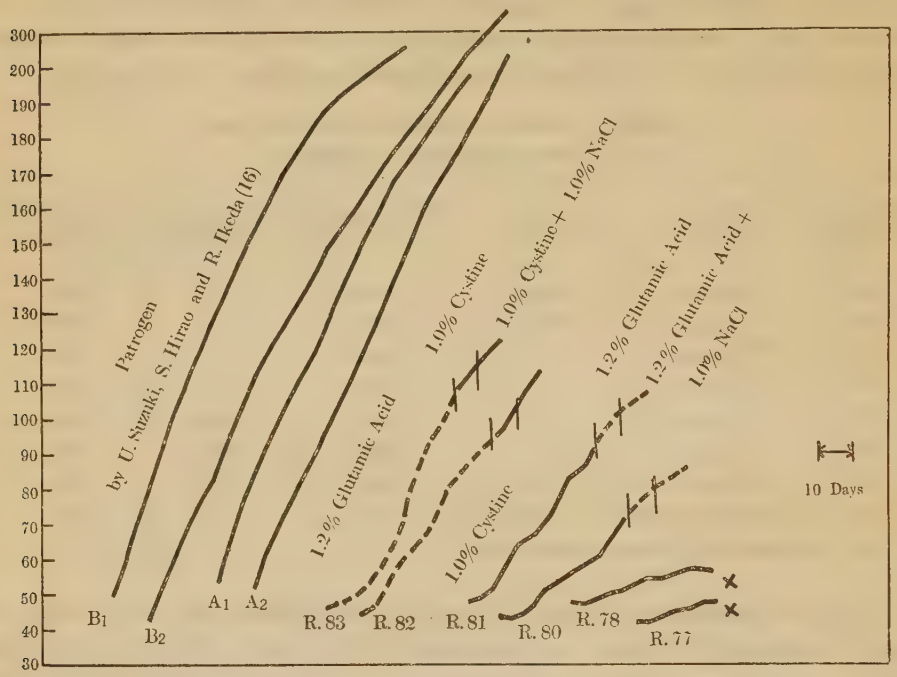

Chart IV.

diet was added 1.0% NaCl in which NaCl was increased from 4.5 to 5.5% of the diet, and the cystine value of the urine was determined as shown in the following table.

TABLE VIII. Influence of Excess of NaCl upon a Rat's Urine.

Days of feeding	Body	Weight (g)	Cystine exc. per day (mg)	c.c. of $N/10$ $AgNO_3$ per day
(A) The first group, "Sendaimiso" diet 1.0% cystine.				
0	43	47	——	——
35	70	88	——	——
36	72	90	2.3	178
37	73	91	2.5	180
38	73	92	2.5	181
"Sendaimiso" diet plus 1.2% glutamic acid				
39	75	95	2.0	190
40	76	96	0.7	195
41	——	——	——	——
42	78	97	0.7	194
43	81	99	0.6	189
44	81	100	0.6	194

The above diet plus 1.0% NaCl.

45	89	102	1.9	125
46	79	103	1.0	214
47	80	102	0.9	225
48	81	104	0.8	220
49				
50	83	105	0.7	235

(B) The second group, "Sendaimiso" diet 1.2% glutamic acid.

0	43	46		
35	91	97		
36	92	102	0.6	170
37	90	103	0.5	185
38	92	105	0.6	167

"Sendaimiso" diet plus 1.0% cystine.

39	93	106	1.2	175
40	93	107	1.8	181
41				
42	98	113	2.1	188
43	99	114	2.0	170
44	100	115	1.8	175

The above diet plus 1.0% NaCl.

45	102	116	1.2	189
46	104	118	1.1	193
47	107	119	0.8	208
48	109	121	0.8	208
49				
50	112	122	0.9	213

These feeding experiments, although of short duration, contain some points of sufficient interest to be recorded. The daily cystine excretion of the animal receiving 1.0% cystine was 2.3～2.5 mg, while that of the animal receiving 1.2% glutamic acid was 0.5～0.6 mg. On the exchange of diets the former showed a rapid decrease in cystine after two days feeding while the administration of 1.0% NaCl gave a temporary rise in the cystine content. The latter showed a slow rise in quantity due to the cystine administration and no change was observed in the amount of NaCl.

Summary.

(1) The content of cystine in various kinds of "Miso" in polished rice, and in soy-beans was determined by Y. Okuda's or by Folin-Looney's method, and whether or not the amount of cystine in the polished rice diet supplemented with "Miso" was sufficient for the growth of white rats was investigated in connection with the excretion of a cystine complex in the urine.

(2) It was found that cystine in "Miso" can be easily decolorized and determined by Okuda's method, after hydrolysing it with concentrated HCl for 12～20 hours. Consequently, the amount of cystine in 100 g of the fresh

"Miso" was as follows:

"Shiromiso" 64.2, "Yedomiso" 45.4, "Sendaimiso" 50.9, and "Hatchomiso" 93.4 mg

(3) The amount of cystine estimated by Folin-Looney's method was slightly larger than the above, asuming "Shiromiso" 67.2, "Yedomiso" 85.6, "Sendaimiso" 86.6, and "Hatchomiso" 103.2 mg.

(4) The cystine content in 100 g of polished rice was 37.8～55.2 mg by Okuda's method.

(5) From the above, the amount of cystine in 100 g of "Miso" diet was calculated to be 0.062～0.065 g and consequently is very slightly defficient in cystine for the growth of white rats, since the diet contains 0.57% cystine for the diet protein.

(6) It was pointed out that the cystine value of the urine, kidneys and livers of the cystine-fed animals were greater than those of the control diet animal, and that the value were probably proportional to the cystine administration. The animal fed on 0.1% cystine diet was approximately normal. The excretion of cystine by the animal placed on 1.0% cystine diet was 4.5 mg per day and was ten times that of the Patrogen fed animal.

(7) Folin-Looney's value of the urine excreted by the animal when fed on an excess of cystine, was temporarily influenced by the excess of NaCl but it gradually diminished.

The author is deeply indebted to Professors Umetaro Suzuki, Bunsuke Suzuki and Rinjiro Sasaki for their encouragement and advice. He also wishes to thank the Suehiro-Miso Manufacturing Company and Dr. S. Yukawa for a sample of "Miso", and Dr. T. Tamura for the gift of *d*-glutamic acid.

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Determination of Cystine in "Shoyu"

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(Received June 16, 1936)

Introduction.

The amount of cystine in "Shoyu", which was made by a fermenting process like that of "Miso", has been determined in connection with the cystine estimation in "Miso". To this case Dr. Y. Okuda's method[1][2] was also applied and the result is given in the following.

Experimental.

For acid hydrolysis, 50 c.c. of "Shoyu" were boiled for 10 hours with 200 c.c. of concentrated hydrochloric acid. This was then filtered, washed with hot water, made up to 250 c.c. To 25 c.c. or 35 c.c. of this hydrolysate, were added 15 c.c. of water, 20% NaOH, and 10 c.c. of a standard cystine solution. Then the solution was decolorized with 0.5 g of Merck's pure animal charcoal, boiled for one minute, filtered by suction, washed about 5 c.c. of water, and reduced by 0.5 g zinc powder. The filtrate was filled up to 100 c.c. and after confirming the fact that the solution was exactly 2% HCl, to 20 c.c. of the solution was added 5 c.c. of both 5% KI and exactly 4% HCl. The mixture was titrated by M/300 potassium iodate (KIO_3) solution, by shaking continuously, until the yellow color fades in exactly one minute.

Cystine was calculated by the formula; cystine (g)$=0.0098/5.1 \times Q$ at 25. Q indicates the reading. Cystine content of several kinds of "Shoyu" is shown in the following table:

Table I. Cystine mg in 100 c.c. of "Shoyu".

Sample	Estimation by mixing with standard Cystine solution	Estimation applied only to the acid hydrolysate
A	42.4	38.5
B	54.4	46.2
C	48.3	42.1
D	49.2	42.2

Summary.

The cystine content in 100 c.c. of "Shoyu" was 42.4～54.4 mg by Y. Okuda's method.

My best thanks are due to Professsors Keijiro Aso, Bunsuke Suzuki and Rinjiro Sasaki for their kind help and encouragement throughout this investigation.

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Beziehungen zwischen Organismenstrahlung, Katalasewirkung und Atmungsintensität bei der Entwicklung von Bombyx mori L.

Von Kazuo YAMAFUJI und Shio GOTO.

(Aus dem Agrikulturchemischen Institut der Kaiserlichen Universität und dem Katakura-Institut für Seidenraupenforschung zu Fukuoka.)

(Eingegangen am 26 Juri, 1936).

Da bei dem Seidenspinner, der nach dem Kokonspinnen ohne Nahrungsaufnahme ein normales Leben führt, während aller seiner Entwicklungsperioden fortwährend tiefgreifende stoffliche und morphologische Umwandlungen stattfinden, bildet der Entwicklungsprozess dieses Insektes einen geeigneten Gegenstand der Forschung des Stoff– oder Energiewechsels im tierischen Organismus. Dies ist einer der Gründe dafür, dass wir gründliche Untersuchungen über die Biochemie der Seidenraupen unternahmen. In einer Reihe diesbezüglicher Arbeiten, welche in japanischen Zeitschriften veröffentlicht wurden und folglich europäischen Forschern schwer zugänglich sind, behandelten wir schon den Einfluss der verschiedenen atmosphärischen Bedingungen auf die Kokonbildung[1], die Veränderungen der Körperbestandteile während der Metamorphose[2] und die Funktionen einiger Enzyme bei den stoffwechselvorgängen im Raupenkörper[3]. Ferner befassen sich die letzten Abhandlungen mit dem Zusammenhang zwischen der sogenannten mitogenetischen Strahlung und den Enzymwirkungen sowohl im Blute als auch in den Eiern[4]. Im folgenden berichten wir über eine Forschung, die diese letzten Angaben ergänzt und einen Beitrag zur Lösung der Problems der physiologischen Funktion der Katalase geben dürfte.

Zu den Versuchen wurden sorgfältig gepflegte gesunde Weibchen und Eier von Bombyx mori verwendet. Um ein genaues Bild der Beziehung der Katalasewirkung zur Organismenstrahlung zu bekommen, wurden die Ermittlungen dieser beiden Phänomen am gleichen Tage angestellt. Die Ergebnisse sind in Fig. 1 zusammengefasst. Von Interesse ist ein Vergleich der erhaltenen Kurven mit den Veränderungen der Atmungsintensität, welche von Kawase[5] und Suzuki[6] verfolgt wurden. Trotzdem der Atemwert der Puppe im Verhältnis zu der Larve und zum Falter äusserst klein ist, hat die Katalase in der jungen Puppenperiode eine viel stärkere Tätigkeit aufzuweisen als in den anderen Perioden. Bemerkenswert ist das Verhalten der Organismenstrahlung. Im Laufe dieser drei Entwicklungsstadien tritt die Strahlenwirkung nur bei der jungen Puppe auf, d. h. in jener Zeit, wo der Katalasegehalt besonders hoch ist. Diese Strahlungserscheinung bei Bombyx mori

stimmt mit dem unlängst von Holzmann[7] festgestellten Rhythmus des mitogenetischen Effekts bei Drosophila melanogaster überein. Was die Eier anbelangt, so ist das praktische Verfahren der Ausbrütung sehr kompliziert. In Fig. 1 zeigt "Ei-(a)" die Resultate der Beobachtungen, die vom Moment der Eierablage des Schmetterlings bis zum Einstellen der Herbstentwicklung der Eier dauerten. "Ei-(b)" stellt die Inkubationsperiode der nach der Überwinterung bei 25° sich belebenden Eier dar. "Ei-(c)" charakterisiert die Entwicklung der durch Salzsäure wiederaufgelebten, annualen Eier, deren Bruttemperatur unveränderlich 25° war. Wie aus den Kurven zu ersehen ist, wurde im Laufe der embryonalen Entwicklungsperiode kein exakter Parallelismus zwischen der Atmungsgeschwindigkeit und der Kalataseaktivität beobachtet.

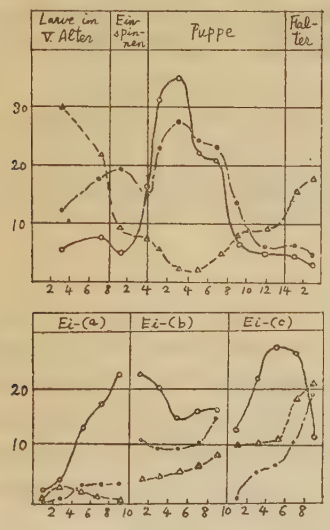

Fig. 1.

○—○ Organismenstrahlung (I).
●—● Katalaseaktivität (II).
△—△ Atmungsintensität (III).
Ordinaten: (I) Induktionseffekt in %; (II) $K \cdot 10^3$; (III) g CO_2 durch 1 kg Tiere in 15 Standen abgegeben.

Dieses Nicht-Übereinstimmen ist aber hierbei nicht so bedeutend wie bei der Metamorphose der Larve in den Falter. Ein gewisser positiver Effekt der Organismenstrahlung kommt nur in bestimmten Zeiten der Entwicklung vor. Das mitogenetische Emissionsvermögen des Eies scheint nicht allein mit der Farbenveränderung im annualen Ei, sondern such mit einer genügend starken Katalaseaktivität und geleichzeitig mit einer in gewissem Grad schwachen Atmungsintensität verknüpft zu sein. In der Periode, wo die Farbe des abgelegten Eies noch gelblich bleibt und der Katalasegehalt verschwindend niedrig ist, sowie auch in dem Moment, in welchem die Raupe aus dem Ei auszukriechen anfängt und die Atmung und Katalasewirkung eine maximal Stärke erreichen, ist das auftreten der Strahlung nicht deutlich. Da wir uns zum Ziel der vorliegenden Arbeit gesetzt hatten, zwischen der Atemgrösse, Katalaseaktivität und dem Strahlungseffekt eine Gesetzmässigkeit zu finden, stellen wir weitere Beschreibung von den Veränderungen derselben ein.

Trotzdem die Erforschung des Mechanismus der Entstehung und Zerlegung von Wasserstoffperoxyd in lebenden Zellen in den letzten Jahren besonders durch die vortrefflichen Untersuchungen von Wieland und seinen Mitarbeitern einen mächtigen Aufschwung durchgemacht hat, dürften unsere Kenntnisse darüber noch lückenhaft sein. Die oben erwähnten Versuche an Bombyx mori geben ein typisches Beispiel dafür, dass im Organismus der

Katalasegehalt nicht immer mit der Atmungsintensität parallel läuft. Was die mitogenetische Strahlung anbetrifft, so sind bisher zahlreiche Angaben publiziert worden, in denen wir eine ganze Reihe zusammenhangsloser und sogar widersprechender Daten auffinden können. Unter Berücksichtigung unserer bisherigen Erfahrungen sind wir der Meinung, dass die Organismen eine Fähigkeit besitzen, solche Strahlen zu emittieren, diese aber nicht stets nachweisbar sind, weil diese Fernwirkung in gewissen Beziehungen zu den Variationen der Zellvogänge steht und die gegenwärtig verwendeten Methoden zur Ermittelung solcher Wirkung noch unvollkommen sind.

Die Versuche sollen in dieser Richtung sowie auch in anderer Beziehung fortgesetzt werden.

Experimenteller Teil.

Die Aufzucht der Seidenraupen wurde im Herbst 1934 mit aller Vorsicht begonnen. Um schwankungen der Lebensvorgänge durch die Temperaturveränderung zu vermeiden, wurde die Zuchttemperatur während der verschiedenen Entwicklungsperioden ungefähr konstant bei 25° gehalten. Die Metamorphose der Larven in die Schmetterlinge verlief normal, und keine Erkrankung wurde beobachtet. Die Belebung der annualen Eier durch Salzsäure führten wir aus, wenn nach der Eiablage vom Falter 20 Stunden abgelaufen waren, jedoch die Farbe der Eier noch unverändert blieb.

Die Ermittelung der Organismenstrahlung geschah durch die Hefenmethode. Zu diesem Zweck wurde die Kojiagarkultur von Saccharomyces ellipsoideus nach dem anweisungen von Baron[8] hergestellt. Nach 16 bis 18 stunden stellten wir die Induktionsversuche an, wobei der Abstand vom Detektor zum Induktor etwa 3 bis 5 mm betrug. Der als Sender benutzte Gewebsbrei wurde in ein Gefäss mit einem Boden aus einer krystallinischen Quarzplatte gebracht, unter welchen die Detektorkultur gestellt wurde. Die Exposition dauerte 10 bzw. 20 Minuten. Die Inkubation der induzierten und Kontrollkulturen, die Zählung der Sprossen und die Berechnung des Induktionseffektes führten wir nach der Vorschrift von Guawitsch[8] durch. Die Ergebnisse in der Fig. 1. stellen die Durchschnittswerte aus mehreren parallelen Versuchen dar. Die Erfahrungen lehrten uns, dass die "feste Hefenmethodik", wenn sie bei günstigen Bedingungen sorgfältig angewandt wird, nicht so unzuverlässig ist, wie sie kürzlich vor allem von Schreiber und Nakaidzumi[9] und von Moissejewa[10] kritisiert wurde.

Die Bestimmung der Katalase erfolgte in der folgenden Weise: Die Eier bzw. Weibchen von Bombyx mori, Quarzsand und 0,2 *m*-Phosphatpuffer von pH=6,8 wurden im Gewichtsverhältnis 1 : 1 : 1 gemischt, zerrieben und mit Wasser 200 fach verdünnt, Diese Mischung brauchten wir als Enzymlösung. Zur Ermittlung der Katalasewirksamkeit wurden 50 ccm 0,01 *n*-H_2O_2-Lösung

mit 2 ccm 0,2 *m*-Phosphatpufferlösung von pH=6,8 und 1 ccm Enzymlösung versetzt. Sofort und nach 5, 10 und 15 Minuten wurden 10 ccm von dieser Reaktionsmischung entnommen und in 5 ccm 5 *n*-H_2SO_4 gegossen. Alsdann titrierten wir das nicht zersetzte H_2O_2 mit 0,01 *n*-$KMnO_4$-Lösung. Die Versuchstemperatur war stets 0°. Die Aktivität der Katalase wurde mit den Reaktionskonstanten erster Ordnung verglichen.

An dieser Stelle danken wir bestens Herrn Prof. Y. Okuda im biologisch-chemischen Institut der Kyushu-Universität für seine freundliche Unterstützung. Die Untersuchungen wurden z. T. mit Mitteln der Japanischen Gesellschaft zur Förderung der Wissenschaften ausgeführt.

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ABSTRACTS

from

TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

On the Unsaponifiable Matter of the Algae Fats. II.—Pelvesterol from *Higikia fusiformis* (Harv.) Okam. (pp. 521～522): By Kiyoshi SHIRAHAMA. (Hokkaido Imperial University; Received Mar, 10, 1936.)

Twenty grams of unsaponifiable matter were separated from 100 g crude fat of *Higikia fusiformis*. These matters contained pelvesterol* (yield of preparation was 9 g) and its properties were as follows:–

	m. p.	$[\alpha]_D$
Sterol	122°	−39.6°
Steryl acetate	118.5°	−44.1°
Steryl propionate	105～106°	−43.1°

The absorption spectrum of pelvesterol ($M \times 10^{-3}$ in C_2H_5OH) showed three narrow bands at 2950, 2825 and 2730 Å, a wide band at 2600 Å and a wider band at 2500 Å.

The author expresses his hearty thanks to Prof. E. Takahashi for his kind leading throughout this work and also he is indebted to Dr. M. Sumi in the Inst. Phys. Chem. Res. for an absorption spectrum.

Chemical Studies on Japanese Coccidae. XII.—On the Carbohydrate and Wax-Substance of Prontapsis yanonensis Kuw. (pp. 523～530): By M. KAWANO and R. MURAYAMA. (Laboratory of Ohsaka Factory of Sankyo Co. Ltd. Received Feb. 27, 1936.)

Studies on "Kaoliang" as the Source of Starch-making, Milling, and "Ame"-Manufacture. (IV.)—Part 2. On Liquefaction- and Saccharification Velocity of Starch by Diastase. (pp. 531～540): By M. NINOMIYA, S. KATAOKA and R. YAMAMOTO. (Central Laboratory, South Manchurian

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Railway Co., Received Mar. 2, 1936,)

Studies on "Kaoliang" as the Source of Starch-Making, Milling and "Ame"-Manufacture. IV.—Part 3. On Maltose-dextrine Ratio in the Saccharificated Products of Starch by Malt-diastase. (pp. 541～547): R. YAMAMOTO and S. MATSUMOTO. (Central Laboratory, South Manchurian Railway Co., Received Mar. 2, 1936.)

Studies on "Kaoliang" as the Source of Starch-Making, Milling and "Ame"-Manufacture. IV.—Part 4. Influences of Heat-treatment under Pressure on the Compositions and Saccharification of Kaoliang. (pp. 548～554): R. YAMAMOTO, S. MATSUMOTO and S. SHIMMI. (Central Laboratory, South Manchurian Railway Co., Received Mar. 2, 1936.)

On the Chemical Constituents of the Bark of Mulberry tree. (I).—*α*-Amyrin. (pp. 555～559): By Masami OKU. (Chemical Laboratory of Gunze Raw Silk Mfg. Co. Ltd., Ayabe-mati, Kyôto-hu, Japan; Received Mar. 4, 1936.)

From a certain fraction of the unsaponifiable matters of petroleum ether extract of the bark of mulberry tree, fine colorless needle shaped crystals were isolated and purified. This substance has the molecular formula $C_{30}H_{50}O$ and melting point 185～186° and belongs to the group of triterpen alcohol. It coincides utterly with *α*-amyrin.

Studies on the Amylase of Yeast. X.—On the Enzyme Chemical Properties. (7). Effect of Mercuric Chloride, Sodium-fluoride, Boiled-Yeast Juice and others on the Action of Yeast-Amylase. (pp. 560～568): By Kazuki OHNO. (Biochemical Laboratory of Taihoku Imperial University, Japan; Received Mar. 4, 1936.

Studies on the Amylase of Yeasts. XII.—On the Enzyme Chemical Properties, (8). Purification of Yeast-Amylase and Properties of the purified Enzyme. (pp. 569～575): By Kazuki OHNO. (Biochemical Laboratory of Taihoku

Imperial University, Japan; Received Mar. 10, 1936.)

Researches on the Amines of Fusel Oil. (pp. 576～582): By Tomotune TAIRA. (Research Lab., Takeda Co. Osaka; Received Mar. 12, 1936.)

A View to the Classification of the Far East and its Nomenclature. (pp. 583～586): By K. SATO. (Laboratory of Brewery, Kotobukiya Co.; Received Mar. 23, 1936.)

The Nutritive value of Pentosan. III.—Decomposition of Xylan by Alimentary-Canal-Bacteria. (pp. 587～594): By Hisayoshi IWATA. (Imperial College of Agriculture and Forestry, Morioka Japan, Received Mar. 16, 1936.)

The xylan decomposing action of micro-organisms living in alimentary canals of men and various higher animals, for examples cattle, horses, sheep, pigs, rabbits, guinea pigs and albino rats were remarkable. As decomposition products, xylose and organic acids such as racemic lactic, butyric, acetic and formic acids were qualitatively and quantitatively determined. The production of carbon dioxide and a very small quantity of hydrogen was also found. Hewever, neither alcohol, aldehyde, methane gas nor any other organic acids were produced. The protein synthesized from xylan and ammonium phosphate, by these micro-organisms, had a similar nature to plant protein.

Studies on the Changes of Chemical Components of the Cucumber with the Nature and Composition of its Sugars. (pp. 595～603): By Kakuo KITAHARA. (Agricultural Chemical Laboratory, Kyôto Imperial University; Received Mar. 12, 1936.)

Studies on the Nodule Bacteria. X.—Influence of some Stimulating Chemicals with Special Reference to the Alkaloids upon the Growth and Morphology of the Nodule Bacteria. (pp. 604～621): By Arao ITANO and Akira MATSUURA. (Ōhara Institute for Agricultural Research, Kurashiki Japan; Received Mar. 18, 1936.)

The influence of ten different alkaloids, three non-alkaloids and yeast water were investigated as to their influence on the growth and morphology of the nodule bacteria. The results are summarized as follows:

1). None of the chemicals tested was effective in stimulating the growth of bacteria in comparison with the yeast water. They in general were rather harmful and more so as the concentration of the chemicals increased.

2). Among the chemicals used, Guanidine, Morphine hydrochloride and Anthraquinone were not harmful for the growth; Pyridine and Strychinine were not so good as the formers; Chinoline was harmful and the growth of all five strains of bacteria was prohibited, while Chinine compounds and Brucine were less harmful.

3). By the addition of chemicals, various forms of bacteria were produced such as rod, coccic, oval, club, branching etc., and in some cases, the granules and vacuoles were produced in the cells. But no correlation was found between the concentration of chemicals and the degree of morphological variation.

4). Caffeine seemed to be the most effective agent in production of large bacteroids in all strains of bacteria; Pyridine, Strychinine, Strychinine nitrate and Chinine compounds were effective in all cases while Guanidine and Morphine hydrochloride were effective only against some special bacterial strains. Among the non-alkaloids. the large bacteroids were found where sodium succinate and emodine were added, and none in cases of Brucine, Anthraquinone and yeast water.

5). As a whole, more of the large bacteroids were found where the growth was poor but not in all the cases.

6). From the foregoing results, it may be stated that the alkaloids have no stimulating effect on the growth of nodule bacteria but they are effective in producing the large bacteroids.

Pulp from Manchurian Cotton Stem. Part I.—(pp. 622～625): Masuzo SHIIKATA Shichiro FUKUWATARI Kazuhiko AKAGI. (Agricultural Chemical Laboratory, Kyôto Imperial University, Received Apr. 5, 1936.)

(1) The chemical constituents of upland cotton stems were given as shown in Table 1.

(2) Soda process combined with dilute alkali pretreatment or dilute nitric acid pretreatment gave a good pulp (α-cellulose content 77～90%) with an yield of 27～30% of the cotton stems.

TABLE 1. Chemical compositions of Cotton Stems.

	Composition \ Sample	Upland cotton (Japan)		Upland cotton (Manchukoku)	
		% for air dry matter	% for abs. day matter	% for air dry matter	% for abs. dry matter
1	Moisture	8.91	——	10.47	——
2	Alcohol-Benzen extractive matter	3.66	4.03	3.31	3.69
3	1% NaOH Soluble M.	28.62	31.44	25.87	28.90
4	Hot water soluble M.	13.01	14.28	7.75	8.66
5	Cold water soluble M.	10.51	11.54	4.82	5.38
6	Crude cellulose	42.78	46.96	43.37	48.45
7*	α-Cellulose	28.88	31.70	31.46	35.15
8	β-Cellulose	13.90	15.26	1.37	1.93
9	γ-Cellulose			10.54	11.37
10	Lignine	20.63	22.63	23.23	25.95
11	Pentosan	18.38	20.18	21.66	24.20
12	Mannan	——	——	——	——
13	Galactan	1.23	1.35	1.03	1.15
14	Hemi-cellulose	19.61	21.53	22.69	25.35
15	CH_3O	3.93	4.31	5.32	5.94
16	Nitrogen	0.77	0.84	0.86	0.96
17	Crude protein	4.81	5.29	5.37	6.00
18	Ash	2.71	2.98	12.06	13.47
19	Ca-pectate	1.82	2.00	1.73	1.93
20	$(CH_3O/Lignin)\times 100$	19.00		22.89	
21	CH_3O in Lignin	——		10.55	

* not corrected with ash and pentosan.

Researches on the Pulp Woods and Rayon Pulp. Part IX.— Chemical Constituents of Woods of Chosen. (pp. 626～628): Masuzo SHIKATA, Akira UMEMURA, Hayao NISHIDA and Nobukiyo URANO. (Agricultural Chemical Laboratory, Kyôto Imperial University, Received Apr. 5, 1936.)

The chemical analyses of woods of chosen were carried out. The results are given in the Table.

TABLE 1.

	Samples	Picea excelsa	Picea jezoensis	Abies nephro-lepis	Pinus koraiensis	Larix dahurica	Tilia amurensis	Betula constate
	Analysed by	Umemura	Umemura	Kino-shita	Tuchi-yama	Urano	Nishida	Nishida
1	Alc.-Benzene Extract	2 52	1.75	4.31	7.11	1.50	5.95	1.53

2	1% NaOH Ext.	11.79	11.65	12.68	15.40	14.95	21.07	20.18
3	Hot water Ext.	5.23	2.65	2.75	7.32	5.43	4.11	1.86
4	Cold water Ext.	1.22	1.33	2.03	5.71	4.65	2.30	0.96
5	Total celluloses	63.33	56.93	55.39	54.08	50.50	61.53	61.33
6	α-Cellulose	43.12	35.34	39.53	38.24	33.35	43.12	38.55
7	β-Cellulose	13.15	12.25	15.86	9.23	5.97	11.97	15.08
8	γ-Cellulose	7.06	9.33		6.68	10.70	6.48	7.94
9	Lignin	29.23	31.71	29.18	28.82	28.05	23.46	21.31
10	Pentosan	12.66	8.82	9.87	9.62	10.28	20.74	26.39
11	Mannan	6.25	7.99	6.89	3.29	1.67	——	——
12	Galactan	1.14	2.29	0.28	4.52	3.27	0.10	0.62
13	Hemicelluloses(10+11+12)	20.05	19.10	17.04	22.43	15.22	20.84	27.01
14	Nitrogene	0.08	0.06	0.12	0.17	0.23	0.11	0.09
15	Crude protein	0.49	0.37	0.75	1.04	1.29	0.69	0.61
16	Ash	0.38	0.28	0.42	0.28	0.49	0.63	0.33
17	CH_3O	5.63	5.92	5.34	4.95	4.96	6.22	5.93
18	CH_3O/Lignin×100	19.26	18.68	18.30	17.18	17.68	26.51	23.03

Researches on the Pulp Woods and Rayon Pulp. Part X.— Chemical Researches on the Manufacture of Rayon Pulp from Hard Woods. (pp. 629～638): Masuzo SHIKATA, Shin-ichi HONDA, Hayao NISHIDA and Megumi SAITO. (Agricultural Chemical Laboratory, Kyôto Imperial University, Received Apr. 5, 1936.)

1. The chemical analyses of poplar (from Hokkaido) and birch (from Hokkaido and Manchukuo) were carried out. The results are given in Table 1.

TABLE 1.

	Samples	Birch (Betula japonica)		Poplar (Populus tremula)	
	Age of Tree	From (Hokkaido) 83	From (Manchukuo) 54	From (Hokkaido) 22	From (Hokkaido) 24
	Analysed by	Honda & Yomo.	Honda & Yomo.	Saito.	Saito.
1	(Moisture)	(9.07)	(12.74)	(13.18)	(13.23)
2	Alc.-Benzen Extract	3.21	2.02	2.11	2.25
3	1% NaOH Ext.	17.96	16.77	16.76	19.11
4	Hot water Ext.	2.09	2.04	2.97	1.50
5	Cold water Ext.	1.74	1.23	1.54	0.17
6	Total Celluloses	57.62	58.17	77.47	75.12
7	α-Cellulose	40.23	38.23	43.77	36.81
8	β-Cellulose	17.39	19.94	1.53	7.42
9	γ-Cellulose			31.02	30.88

10	Lignin	18.84	19.20	18.74	20.53
11	Pentosan	26.09	26.54	18.72	23.58
12	Mannan	——	——	0.05	0.02
13	Galactan	0.68	0.72	1.00	0.29
14	Hemicelluloses(11+12+13)	26.77	27.26	19.77	23.89
15	Nitrogen	0.10	0.08	0.10	0.08
16	Crude protein	0.64	0.54	0.62	0.51
17	Ash	0.45	0.41	0.61	0.81
18	CH_3O	6.28	7.31	5.64	5.94
19	CH_3O/Lignin×100	33.33	38.09	30.18	28.92
	In total celluloses				
	α-Cellulose	69.82	65.72	57.98	49.00
	β-Cellulose	30.18	34.28	1.98	9.88
	γ-Cellulose			40.04	41.12

2. The soda pulp was prepared from the Manchurian as well as Japanese birch.

3. The systematic experiments with regard to the cooking time and main digestion temperature were carried out.

4. With the main digestion temperature of 160°C, 1 to 2 hours were sufficient in order to obtain good pulp (-cellulose content over 88%) with the good yield (45%).

5. With the main cooking time of 6 hours, the 150～160°C is sufficient in order to get good pulp.

On the Acetone-butanol Fermentation. IX.—Isolation of Organisms. (pp. 639～649): By Bunzo ROKUSHO. (The Central Laboratory of South Manchurian Railway Co.; Received Apr. 13, 1936.)

Twelve years ago (1924) I isolated an organism according to the method similar to that described by Weizmann from corn grown in Shizuokaken, Japan which produce acetone and butyl alcohol, and the results of works with the organism has been reported several times in the preceeding papers.

In this paper the results of experiments on morphological and cultural study of this organism are described.

Morphology. Vegetative cells, medium 5% rice mash, temperature 37° C, age 24 hours: Form rods, size $0.7 \sim 1.0 \times 2.8 - 4.2\ \mu$, age 40 hours: Size $1.4 \times 5.6\ \mu$, motile, granulose positive, gram positive. Age 70 hours: spore is formed in the center. Spore size $1.4 \times 2.8\ \mu$, clostridia $1.4 \times 4.2\ \mu$.

Cultural characteristics. This organism is anaerobic, optimum temperature for growth and fermentation is 37～39°C. Spore is resistant to heat. This

organism ferment glucose, lactose, sucrose, levulose, galactose, mannose, arabinose, xylose, soluble starch, pectin, mannitol, glycogen, dextrine, melezitose, salicin, amygdalin, α-methyl-glucoside, maltose, but not ferment ca-lactate, inulin, glycerol, isodulcit, trehalose, melibiose. This organism ferment starchy materials such as corn, rice, milet and produce 10～11% acetone, 20～22% butyl alcohol and a little ethyl alcohol, H_2, CO_2 and acids from starch, but it is hard to ferment kaoliang containing rich in tannic substance. But the unfermentability is cured by a little addition of proteins, peptones, some amino acids and ammonium salts.

Nitrogen requirements. This organism can assimilate protein (vegetable or animal origin), peptone, amino acid (mixed or single such as glutamic acid) and ammonium salt (such as ammonium acetate.)

Reduction. This organism reduce nitrite, Na_2SO_3, $Na_2S_2O_3$ and dye stuff such as methylen blue, methyl red and gentiana violet B.

Acethyl methyl carbinol formation. This organism produce acethyl carbinol in a small amount.

Hydrogen sulfide production. H_2S is formed during fermentation of corn, rice, oat, but not from kaoliang.

From the results of experiments this organism seems to be a strain of clostridium acetobutylicum.

Recently I attempted to isolate acetone producing organisms from cereals, soils and other materials, about 30 samples collected in corea and 90 samples collected in Manchuria, but only 5 cases were succeeded in a pure state from kaoliang grown in Manchuria. These isolated organisms produce 6～7.5% acetone, 13.6～15.5% butylalcohol and 2.3～2.9% ethyl alcohol from rice.

Studies on the Amylase of Yeast. XII.—On the Action of Yeast Amylase in Macerations-juice and Some Enzyme Solution which reported in a Previous Papers (8). (pp. 650～660): By Kazuki ONO. (Biochemical Laboratory of Taihoku Imperial University, Japan; Received Apr. 13, 1936.)

Über kahmhefe-verhütenden Bestandteil im unverseifbaren Anteil von Soja-öl, Tamariöl und Tamarikoji-öl. (S. 661～674): von Kenji MIYAJI und Motozo OGURI. (Landwirtschaftliche Hochschule zu Gifu; Eingeg. am 16, April, 1936.)

Chemische Untersuchung über die Bestandteile der Rosskastaniensamen.—I. Mitteil. Über den Nährwert. (S. 675～683): von Dr. R. SASAKI und M. KANDATSU. (Aus der Agrikulturchem, Institut d. Univers. Tokio; Eingeg. am 27, März, 1936.)

Es ist nach verf. Tierversuch (Ratte albino) angegeben worden, dass Pulver von ungeschält gepulverten Rosskastaniensamen (Aesculus turbinata, Blume.) als Lebensmittelersatz nicht brauchbar sind, weil sie mehrere glukosideartige substanzen enthalten sind. Aber wenn sie mit verdünnter Alkalikarbonate gewäscht werden, ergeben sie keine giftige Erscheinungen über die Ernährungszustände von Ratten und können ihr normales Wachstum erhalten. So kann man obige-, alkali behandelten Pulver von Rosskastaniensamen ganz genügend ersetzlich brauchbar machen.

On an Active Biological Factor in the Cow's Milk. (pp. 684～698): By Rinziro SASAKI and Norihide ANDO. (Agricultural Chemical Laboratory, Tokio Imperial University; Received Mar. 27, 1936.)

By the biological experiment of some milk product, the presence of an active component in the cow's milk was observed.

In our experiments the albino rats, when they were supplied excess calcium in bread ration, became very poor appearance in the hair coat. The special biological factor in the cow's milk has the activity to cure this poor condition.

But it is necessary to confirm this activity of milk in further investigation under more careful treatment.
